UTILITY PATENT APPLICATION

TITLE OF INVENTION: **MICROFABRICATION OF POLYMER MICROPARTICLES**

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TITLE OF THE INVENTION

MICROFABRICATION OF POLYMER MICROPARTICLES

CROSS - REFERENCE TO RELATED APPLICATIONS

This patent application claims the benefit of U.S. Provisional Patent Application Serial No. 60/408,557 filed on September 6, 2002 and entitled "Microfabrication of Polymer Microparticles," the disclosure of which is incorporated as if fully rewritten herein.

STATEMENT REGARDING FEDERALLY FUNDED R&D

This invention was made with government support under Agreement Number F30602-00-2-0613 awarded by the Defense Advanced Research Projects Agency (DARPA), and the Air Force Research Laboratory (AFRL), Air Force Materiel Command, USAF. The government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to methods and techniques for fabricating microparticles for a use in scientific and/or medical applications and more specifically to a microfabrication method for creating polymer microparticles having certain geometric, structural, and compositional characteristics.

BACKGROUND OF THE INVENTION

Polymer microparticles are useful for a variety of applications, including biological and medical analysis, drug delivery, bio-separation, and clinical diagnosis. Polymer microparticles may take numerous forms such as, for example, microbeads, microspheres, microbubbles, and/or microcapsules. A variety of manufacturing and/or fabrication methodologies have been developed and applied to create such microparticles. These known methods include spray drying, phase separation, and emulsification. Despite the demonstrated effectiveness of these techniques, the microparticles produced by these methods are typically limited to shapes that are spherical or substantially spherical. Furthermore, the size of the particles produced by these methods is often widely distributed.

While spherical microparticles are useful for certain applications such as drug delivery, non-spherical particles may prove to have more desirable characteristics. Substantially flat microparticles possess a comparatively large surface area and, as will be appreciated by those skilled in the art, may be more suitable for cell or tissue binding applications. Furthermore, discrete control of particle geometry, and other characteristics such as particle thickness, may facilitate more precise bio-analysis and controlled drug delivery because the shape of a particle can be tailored to function more effectively under certain predefined conditions. Thus, there is a need to identify an effective method for fabricating substantially flat microparticles having desired geometries, structural characteristics, and/or other characteristics that provide enhanced functionality in various applications.

Microfabrication techniques conventionally used for making integrated circuits have recently been utilized to create microparticles by combining silicon dioxide or polymethylmethacrylate (PMMA) and a photo-sensitive polymer. These techniques can be used to create microparticles having a precise shape, uniform size and specifically designed structures and surface chemistries, thereby making them suitable for use as drug-carrying vehicles. However, these techniques are limited in that they (i) require the use of photolithography to create every particle and (ii) are compatible with only certain materials. Moreover, the rigorous conditions, including highly aggressive solutions and elevated temperatures, which are used to release fabricated microparticles into solution may damage fragile compounds that have been incorporated into the microparticles. Thus, there are significant limitations to using known photolithographic techniques for microfabrication of shaped microparticles.

An alternative to conventional photolithographic techniques is soft-lithography. Soft lithography is a collective term that refers to a group of non-photolithographic microfabrication techniques that employ elastomeric stamps having certain three dimensional relief features to generate micro-structures and even nano-structures. A more detailed description of soft lithography is found in Xia and Whitesides, *Annual Review of Materials Science* 28: 153-84 (1998) incorporated herein by reference. Thus, there is a need to utilize such alternate microfabrication techniques to create polymer microparticles having certain desired geometries.

SUMMARY OF THE INVENTION

These and other deficiencies of the prior art are overcome by the present invention, which provides a system and methods for using common thermoplastic polymers to prepare thin-film microparticles having well-defined lateral geometries and other desired characteristics. The components of the exemplary system include a PDMS stamp having micro-contours or micro-structures, a substrate, and a sacrificial layer of material coating the substrate. The basic method includes the steps of coating the face of stamp with a thin layer of polymer to cover the micro-structures of the stamp, contacting the coated face of the stamp with the coated glass slide to transfer polymer from the micro-structures of the stamp to the slide to create free-standing polymer microparticles, and dissolving the sacrificial layer covering the substrate to release the microparticles into solution. The microparticles fabricated by this method typically exhibit well-defined geometries that correspond to the micro-structures of the stamp.

Further advantages of the present invention will become apparent to those of ordinary skill in the art upon reading and understanding the following detailed description of the preferred embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and form a part of the specification, schematically illustrate one or more exemplary embodiments of the invention and, together with the general description given above and detailed description of the preferred embodiments given below, serve to explain the principles of the invention.

FIGS. 1*a-d* illustrate graphically the system components and stepwise method of the embodiment of the microfabrication technique of the present invention that utilizes the micro-pillar surface structures of a PDMS stamp.

FIGS. 2*a-f* illustrate graphically the system components and stepwise method of the embodiment of the microfabrication technique of the present invention that utilizes the micro-well surface structures of a PDMS stamp.

FIGS. 3*a-e* illustrate graphically the system components and stepwise method of the embodiment of the microfabrication technique of the present invention that utilizes a discontinuous wetting technique to fill the well surface structure of a PDMS stamp.

FIGS. 4*a-h* illustrate graphically the system components and stepwise method of the embodiment of the microfabrication technique of the present invention that utilizes the multiple filling process to produce multi-layer particles within the well surface structures of a PDMS stamp.

FIG. 5*a* is an optical micrograph of polymer microparticles attached to a substrate, showing replication of the geometry of the micro-pillars found on the face of the PDMS stamp.

FIG. 5*b* is an optical micrograph of the microparticles of FIG. 5*a* released from the substrate and floating freely in solution.

FIG. 6*a* is an optical micrograph of polymer microparticles attached to a substrate, showing replication of the geometry of the micro-wells found on the face of the PDMS stamp.

FIG. 6*b* is an optical micrograph of the microparticles of FIG. 6*a* released from the substrate and floating freely in solution.

FIG. 7 is an optical micrograph of microparticles fabricated using the discontinuous wetting technique floating freely in solution after release from the substrate.

FIG. 8 is an optical micrograph of 3-layer microparticles fabricated using the multiple layer technique floating in solution, showing the middle layer of FSPAN swollen, but confined between the two layers of PPMA.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a basic system and several alternate methods for using common thermoplastic polymers to prepare thin-film microparticles that exhibit well-defined lateral geometries and other desired characteristics. The exemplary embodiment of this

system includes a polydimethyl siloxane stamp having micro-contours or micro-structures, a substrate, and a sacrificial layer of material coating the substrate. As described below, stamps with both isolated protruding structures and recessed structures can be used to create polymer microparticles using the system and methods of this invention.

The exemplary methods of the present invention primarily utilize the polymer polypropyl methacrylate ("PPMA"), although other common polymers such as, for example, polylactic-co-glycolic acid, polycaprolactone, polymethyl methacrylate, and polystyrene have been successfully demonstrated with this system. Furthermore, the general methods disclosed herein are easily extendable to most polymers, and thermoplastic polymers, in particular. The exemplary system also utilizes polydimethyl siloxane (PDMS) stamps having two different types of surface structures: (i) micro-pillars, which comprise square-like members with rounded corners protruding from the face of the stamp, and (ii) micro-wells which comprise square-like recessed areas formed between the micro-pillars on the face of the stamp. As will be appreciated by those skilled in the art of soft-lithography, PDMS stamps are typically created from molds. The dimensions of PDMS stamps are typically about 1.0 cm x 1.0 cm, although much larger stamps can be created for large-scale manufacturing.

In exemplary embodiment, the sacrificial layer component typically consists of polyvinyl alcohol (PVA) due to its solubility in water and its high melting temperature. However, other materials that exhibit solubility in water and relatively low solubility in other solvents may be suitable for the disclosed system. In other embodiments, water-soluble inks, glucose, chitosan, and polyethylene glycol (PEG) are utilized. In the exemplary methods, the substrate that the sacrificial layer is deposited on is typically a glass slide; however, other substantially flat, smooth, non-porous materials may be used.

I. Micro-Pillar Printing Method

With reference now to FIG. 1*a-d*, a first embodiment of microfabrication system **100** includes a stamp **102**, a substrate **112**, and a water-soluble sacrificial layer **110**. Utilizing system **100**, microparticles **114** are fabricated according to the following exemplary method:

(i) dip stamp **102** into a 5.8 wt % PPMA/acetone solution to form a thin, continuous layer **108** of PPMA on the face of the stamp (see FIG. 1*a*) which covers its contours, i.e., micro-pillars **104** and micro-wells **106**;

(ii) using a cotton swab or other suitable applicator, brush a 1.5 wt % aqueous solution of polyvinyl alcohol (PVA) onto the surface of a glass slide (substrate **112**) to form a thin film which will serve as sacrificial layer **110** (see FIG. 1*b*);

(iii) place stamp **102** on substrate **112** with the polymer-coated face touching the surface of the slide and sacrificial layer **110** and place a solid weight on top of the stamp, creating a pressure of about 320Pa, to ensure a complete conformal contact between stamp **102** and substrate **112**;

(iv) place the weight, stamp, and slide on a hot plate at about 110°C for about ten seconds;

(v) peel stamp **102** away from substrate **112** leaving the polymer microparticles attached to sacrificial layer **110** (see FIG. 1*c*);

(vi) place substrate **112** into water-filled reservoir **116** to dissolve sacrificial layer **110** and release microparticles **114** into solution (see FIG. 1*d*); and (optionally)

(vii) retrieving microparticles from solution by means of desiccation, filtration, or any other suitable method.

With reference to FIG. 5*a*, the width and height of micro-pillars **104** is about 30μm by about 3.7μm, respectively, and the resultant particles have a width of about 30μm and thickness of about 650nm. FIG. 5*a* is an optical micrograph of microparticles **114** on substrate **112** showing replication of the structures of the micro-pillars, namely the generally square shape with rounded corners. FIG. 5*b* is an optical micrograph of microparticles **114** released into a solution of water after sacrificial layer **112** has been dissolved.

II. Micro-Well Printing Method

With reference to FIG. 2*a-f*, a second embodiment of this invention, microfabrication system **200**, uses a stamp **202**, a substrate **212**, and a water-soluble sacrificial layer **210** to create polymer microparticles **214**. Utilizing system **200**, microparticles **214** are fabricated according to the following exemplary method:

(i) stamp **202** is dipped into a 2.5 wt % PPMA/acetone solution to form a thin, continuous layer **208** of PPMA on the face of the stamp (see FIG. 2*a*) and covering its contours, i.e., micro-pillars **204** and micro-wells **206**;

(ii) place the polymer-coated stamp on a glass slide **212** (see FIG. 2*b*) and apply pressure of about 550Pa to the stamp using a solid weight to induce a full conformal contact between the polymer on the raised regions of the stamp and the glass slide across the entire face of the stamp (note: care should be taken to not apply excessive pressure resulting in deformation of the stamp that would allow the polymer in micro-wells **206** to be transferred to the slide);

(iii) place the weight, stamp, and slide on a hot plate at about 110°C for about ten seconds, and then remove the polymer-coated stamp from the glass slide (see FIG. 2*c*) leaving the excess polymer **211** that coated micro-pillars **204** on the surface of the slide (note: the polymer deposited on the slide in this step is no longer needed and this slide may be discarded or recycled after this step);

(iv) using a cotton swab or other suitable applicator, brush a 1.5 wt % aqueous solution of polyvinyl alcohol (PVA) onto the surface of a second glass slide (new substrate **212**) to form a thin film which will serve as sacrificial layer **210**;

(v) place stamp **202** on substrate **212** with the polymer-coated face touching the surface of the slide and sacrificial layer **210**, and for about five seconds place a solid weight or other suitable compression means on top of the stamp (creating a pressure of greater than about 2.5 kPa) to push the polymer in micro-wells **206** onto substrate **212** (see FIG. 2*d*);

(vi) place the weight, stamp, and slide on a hot plate at about 110°C for about ten seconds, and then remove the polymer-coated stamp from the glass slide (see FIG. 2*c*) leaving the polymer that coated micro-wells **206** on the surface of the slide attached to sacrificial layer **210** (see FIG. 2*e*);

(vii) place substrate **212** into water-filled reservoir **216** to dissolve sacrificial layer **210** and release microparticles **214** into solution (see FIG. 2*f*); and (optionally)

(viii) retrieving microparticles from solution by means of desiccation, filtration, or any other suitable method.

With reference to FIGS. 6*a-b*, the stamp used in this embodiment includes 40um-wide square micro-wells separated by 10um-wide ridges which are about 1.4μm height. The microparticles created by this exemplary method have an average thickness of about 130nm; however, the rims or outer edges of these microparticles may be as thick as about 300nm to 600nm. FIG. 6*a* is an optical micrograph of microparticles **214** as they appear on the surface of substrate **212**. The square-like shape of the microparticles is clearly evident in FIG. 6*a*. FIG. 6*b* is an optical micrograph of microparticles **214** released into a solution of water after sacrificial layer **212** has been dissolved.

III. "Discontinuous Wetting" Method

With reference to FIG. 3*a-e*, a third embodiment of this invention, microfabrication system **300**, uses a stamp **302**, a substrate **312**, and a water-soluble sacrificial layer **310** to create polymer microparticles **314**. Utilizing system **300**, microparticles **314** are fabricated according to the following exemplary method:

(i) apply 10 wt % poly(lactic-glycolic)acid (PLGA)/ dimethyl sulfoxide (DMSO) solution to stamp **302** to fill only the micro-well features (see FIG. 3*a*);

(ii) evaporate the solvent (DMSO) under vacuum overnight, leaving PLGA solid polymer **308** in the micro-well features on the face of the stamp (see FIG. 3*b*);

(iii) using a cotton swab or other suitable applicator, brush a 1.5 wt % aqueous solution of polyvinyl alcohol (PVA) onto the surface of a glass slide (new substrate **312**) to form a thin film which will serve as sacrificial layer **310**;

(iv) place stamp **302** on substrate **312** with the polymer-coated face touching the surface of the slide and sacrificial layer **310**, and for about five seconds place a solid weight or other suitable compression means on top of the stamp (creating a pressure of greater than about 2.5 kPa) to push the polymer in micro-wells **306** onto substrate **312** (see FIG. 3*c*);

(v) place the weight, stamp, and slide on a hot plate at about 110°C for about ten seconds, and then remove the polymer-coated stamp from the glass slide leaving the polymer that coated the micro-wells **306** on the surface of the slide attached to sacrificial layer **310** (see FIG. 3*d*);

(vi) place substrate **312** into water-filled reservoir **316** to dissolve sacrificial layer **310** and release microparticles 3**14** into solution (see FIG. 3*e*); and (optionally)

(vii) desiccate, filter, or use other conventionally accepted methods to retrieve microparticles from solution.

With reference to FIG. 7, the stamp used in this embodiment includes 40um-wide square micro-wells separated by 10um-wide ridges which are about 1.4μm height. FIG. 7 is an optical micrograph of microparticles **314** released into a solution of water immediately after sacrificial layer **312** has been dissolved, still floating loosely above their original positions on the substrate. This technique can be used for solution casting as described above with the appropriate solvent/ stamp combination, or also for casting and curing a pre-polymer solution such as methacrylic acid (MAA) for the formation of cross-linked microparticles (PMAA, a hydrogel).

IV. "Multi-Layer" Method

With reference to FIG. 4*a-h*, a fourth embodiment of this invention, microfabrication system **400**, uses a stamp **402**, a substrate **412**, and a water-soluble sacrificial layer **410** to create polymer microparticles **414**. Utilizing system **400**, microparticles **414** are fabricated according to the following exemplary method:

(i) stamp **402** is dipped into a 2.5 wt % PPMA/acetone solution to form a thin, continuous layer **408** of PPMA on the face of the stamp (see FIG. 4*a*) and covering its contours, i.e., micro-pillars **404** and micro-wells **406**;

(ii) place the polymer-coated stamp on a glass slide **412** (see FIG. 4*b*) and apply pressure of about 550Pa to the stamp using a solid weight or other suitable compression means to induce a full conformal contact between the polymer on the raised regions of the stamp and the glass slide across the entire face of the stamp (note: care should be taken to not apply excessive pressure resulting in deformation of the stamp that would allow the polymer in micro-wells **406** to be transferred to the slide);

(iii) place the weight, stamp, and slide on a hot plate at about 110°C for about ten seconds, and then remove the polymer-coated stamp from the glass slide leaving the excess polymer **411** that coated micro-pillars **404** on the surface of the slide (note: the polymer deposited on the slide in this step is no longer needed and this slide may be discarded or recycled after this step);

(iv) brush fully sulfonated polyaniline (FSPAN) / DMSO solution onto stamp **402** to form spots of solution within the microwell features **406** on top of the previously deposited PPMA;

(v) evaporate DMSO solvent in vacuum overnight, leaving solid polymer FSPAN on top of PPMA within the microwells **406** (see FIG. 4*c*);

(vi) dip stamp **402** into a 2.5 wt % PPMA/acetone solution to form a thin, continuous layer **408** of PPMA on the face of the stamp (see FIG. 4*d*) that is bonded to the first layer of PPMA (see FIG. 4*d*);

(vii) repeat steps (ii) and (iii) to remove excess polymer **411** that coats the micro-pillars **404** onto the surface of the slide (see FIG. 4*e*);

(viii) using a cotton swab, brush a 1.5 wt % aqueous solution of polyvinyl alcohol (PVA) onto the surface of a new glass slide (new substrate **412**) to form a thin film which will serve as sacrificial layer **410**;

(ix) place stamp **402** on substrate **412** with the polymer-coated face touching the surface of the slide and sacrificial layer **410**, and for about five seconds place a solid weight or other suitable compression means on top of the stamp (creating a pressure of greater than about 2.5 kPa) to push the polymer in micro-wells **406** onto substrate **412** (see FIG. 4*f*);

(x) place the weight, stamp, and slide on a hot plate at about 110°C for about ten seconds, and then remove the polymer-coated stamp from the glass slide leaving the p olymer that coated micro-wells **406** on the surface of the slide attached to sacrificial layer **410** (see FIG. 4*g*);

(xi) place substrate **412** into water-filled reservoir **416** to dissolve sacrificial layer **410** and release microparticles **414** into solution (see FIG. 4*h*); and (optionally)

(xii) desiccate, filter, or use other conventionally accepted methods to retrieve microparticles from solution.

With reference to FIG. 8, the stamp used in this embodiment includes 40um-wide square micro-wells separated by 10um-wide ridges which are about 1.4μm height. The microparticles created by this exemplary method demonstrate the multi-layer properties through the swelling of the confined FSPAN layer which is completely encapsulated between the two PPMA layers. FIG. 8 is an optical micrograph of microparticles **414** released into a solution of water after sacrificial layer **412** has been dissolved and the interior FSPAN layer

has swollen. This technique can be used to produce microparticles of any multitude of layers for added functionality, so long as the cumulative thickness of the microparticles is less than the micro-well depth on the PDMS stamp.

All embodiments of the system and method of the present invention enable microfabrication of geometrically uniform microparticles over relatively large surface areas on the substrate. Optical profilometry can be employed to confirm that these microparticles have the same lateral sizes as the stamp structures for both the micro-pillar method and micro-well methods. Optical profilometry can also be used to confirm that microparticles made with the micro-pillar method are typically thicker in the center portion of the particle, while the microparticles made with micro-well method typically include a thin central portion but have a thicker rim portion.

While the exemplary methods disclosed herein include the use of stamps with square-like structures, stamps or other templates having any number of different geometries can be used to create polymer microparticles. Thus, polymer microparticles having any variety of lateral shapes can be produced with these methods provided that a continuous film of polymer is formed on the face of the stamp such that it covers the micro-structures or micro-contours of the stamp. In some embodiments that utilize different stamps or templates, the concentration of the polymer solution for dip coating may have to be adjusted to achieve optimal film formation. In general, the thickness of the film and of the resultant microparticles, is proportional to the concentration of the solution. Thus, for different polymers or combinations of polymers, optimal concentrations should be determined empirically. Likewise, polymers other than those described in the exemplary methods will have different thermal and cross-linking properties; therefore, system parameters such as temperatures and exposure times may need to be adjusted accordingly.

The systems and methods disclosed basically fall into to broad categories, namely the "micro-pillar" technique and the "micro-well" technique. Although closely related, each technique has its own particular applications and advantages. For example, the micro-pillar printing technique, is essentially a one-step process which is simplistic and relative easy to perform. This one-step process may be repeated using the same stamp and the same substrate to create polymer structures having multiple layers. Each new layer added to the first layer of

polymer may include the same or different polymer(s) and the same or different shapes, patterns, geometries, or other desired characteristics. The micro-well printing technique is essentially a two-step process that includes an additional printing step to remove unneeded polymer film on the ridges of the stamp before printing out the microparticles on the substrate.

The micro-well printing technique can also be used to fabricate multi-layered microparticles by filling the micro-wells multiple times and transferring the polymer to the substrate to create composite microparticles. The discontinuous wetting and the multi-layered method described above are embodiments of the present invention that incorporate the micro-well technique. Advantageously, the micro-well method may also be performed partially in the absence of elevated temperature, which is only needed to remove polymer between the micro-wells in the first printing. The second printing, which transfers polymer in the micro-wells onto the sacrificial layer, can be carried out at room temperature simply by making the sacrificial layer tacky, which is easily achieved through a brief exposure of a dry PVA layer to hot water vapor.

It should also be noted, that while the sacrificial layer of material is included as a component in the described system and methods, all of the methods described herein can be performed without this sacrificial layer to create microparticles that remain attached to the substrate material following the various printings.

While the above description contains many specificities, these should not be construed as limitations on the scope of the invention, but rather as exemplification of certain preferred embodiments. Numerous other variations of the present invention are possible, and is not intended herein to mention all of the possible equivalent forms or ramifications of this invention. Various changes may be made to the present invention without departing from the scope or spirit of the invention.